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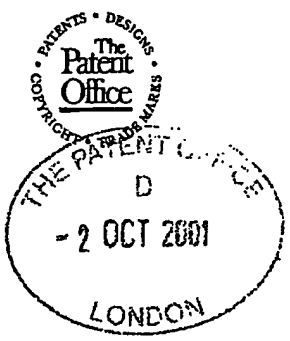


1/77

## Request for grant of a patent

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1. Your reference — P012606GBR NJN

2. Patent application number *(The Patent Office will fill in this part)* — 02 OCT 2001 0123629.8

03DC101 E664214-4 D02246
P01/7700 0.00-0123629.8

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Cyclacel Ltd
   Dundee Technopole,
   James Lindsay Place,
   Dundee,
   DD1 5JJ, Scotland

   07718467001
   Patents ADP number *(if you know it)*

   If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — ANTICANCER COMPOUNDS

5. Name of your agent *(if you have one)* — D Young & Co

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   21 New Fetter Lane
   London
   EC4A 1DA

   Patents ADP number *(if you know it)* — 59006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   *a) any applicant named in part 3 is not an inventor, or*
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| Priority documents | |
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11. I/We request the grant of a patent on the basis of this application.

Signature D Young & Co Date 02 October 2001

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Neil Nachshen — 020 7353 4343

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Anti-cancer compounds

The present invention relates to 2-substituted 4-heteroaryl-pyrimidines, their preparation, pharmaceutical compositions containing them, and their use in the treatment of proliferative disorders such as cancer, leukemia, psoriasis and the like.

**Introduction and Summary of the Prior art**

Certain 4,5,6-substituted-N-(substituted-phenyl)-2-pyrimidineamines having anti-asthmatic properties are disclosed in EP-A-233,461. Certain 4-heteroaryl-N-(3-substituted-phenyl)-2-pyridineamines possessing anti-proliferative properties and inhibiting protein kinases C, epidermal growth factor receptor-associated tyrosine protein kinase (EGF-R-TPK), as well as CDK1/cyclin B have been disclosed in WO95/09847 wherein the exemplified heteroaryl groups are pyridyl and indolyl.

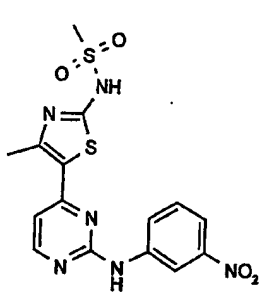
J. Med. Chem. (1993) Vol. 36, pages 2716-2725, Paul, R. et al: discloses a further class of phenyl amino-pyrimidines possessing anti-inflammatory activity. These compounds include mono-substituted 2-thienyl groups at the 4-position of the pyrimidine ring and dimethyl-3-furyl groups at this position.

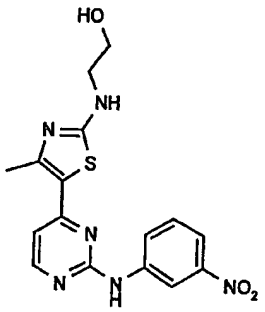
PCT/GB01/01423 discloses a broad range of 2-subtituted 4-heteroaryl-pyrimidines which inhibit cyclin-dependent kinases (CDKs) and have applications in the treatment of proliferative disorders such as cancer, leukaemia, psoriasis and the like.

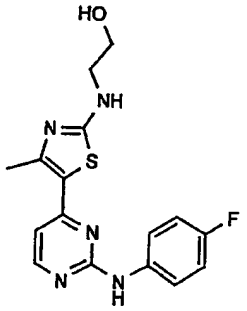
It is an aim of the present invention to provide further N-phenyl-2-pyrimidine anti-proliferative compounds. The compounds of the present invention have surprisingly been found to not to be inhibitors of protein kinase C. As discussed hereinafter, their activity may be demonstrated by inhibition of cell proliferation in cell lines and/or inhibition of cyclin dependent kinase enzymes.

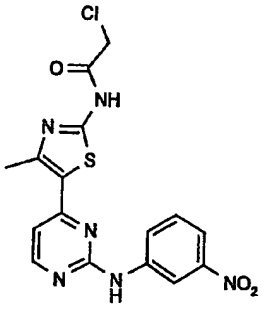
## Summary of the Invention

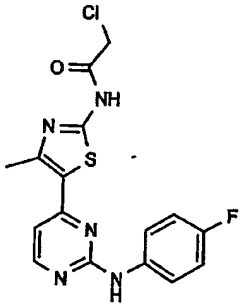
In a first aspect, the present invention relates to compounds having the following formulae:

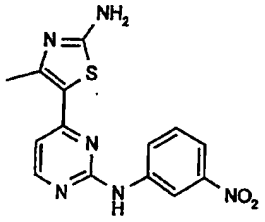


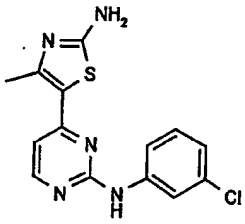


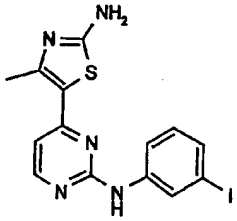


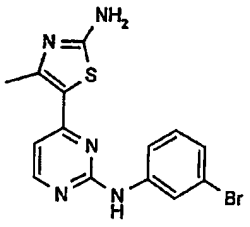


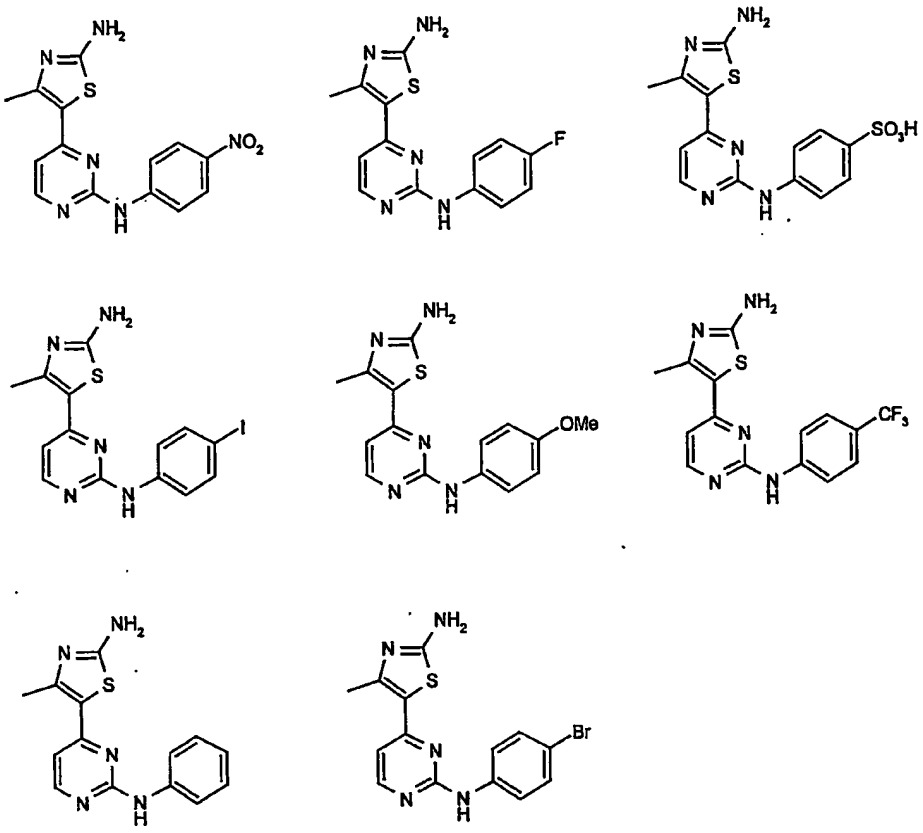












and pharmaceutically acceptable salts thereof.

In a second aspect, the invention provides a pharmaceutical composition comprising one or more of said compounds together with a pharmaceutically acceptable carrier, excipient or diluent.

In a third aspect, the invention relates to the use of one or more of said compounds in the treatment of a proliferative disorder.

## Detailed Description

In a preferred embodiment, the compound of the invention is:
[4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine;
*N*-{4-methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-yl}-methanesulfonamide; or
2-{4-methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-ylamino}-ethanol.

In a particularly preferred embodiment, the compound is [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine.

The compounds of the invention have been found to possess anti-proliferative activity and are therefore believed to be of use in the treatment of proliferative disorders such as cancers, leukaemias and other disorders associated with uncontrolled cellular proliferation such as psoriasis and restenosis. As defined herein, an anti-proliferative effect within the scope of the present invention may be demonstrated by the ability to inhibit cell proliferation in an *in vitro* whole cell assay, for example using any of the cell lines A549, HT29, Saos-2, HeLa or MCF-7, or by showing inhibition of a CDK enzyme (such as CDK2 or CDK4) in an appropriate assay. These assays, including methods for their performance, are described in more detail in Example 7. Using such cell line and enzymes assays it may be determined whether a compound is anti-proliferative in the context of the present invention.

Without wishing to be bound by theory, the compounds of the present invention are believed to exert their anti-proliferative effect in a non-protein kinase C (PKC) dependent manner. Many of the compounds inhibit cyclin-dependent kinase enzymes (CDKs) that have been shown to be involved in cell cycle control. These CDKs include CDK2 and CDK4 and particularly their respective interactions with cyclin E and cyclin D1. These compounds of the present invention are further believed to be advantageous in being selective for CDK enzymes implicated in proliferative diseases.

By the term "selective" it is meant that although possibly having some inhibitory effect on another enzyme (such as PKC), the compound is preferentially effective against an enzyme implicated in proliferative diseases.

The compounds of the invention may inhibit any of the steps or stages in the cell cycle, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, and cytokinesis functions. In particular, the compounds of the invention may influence certain gene functions such as chromatin binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

One embodiment of the present invention therefore relates to the use of one or more compounds of the invention in the treatment of proliferative disorders. Preferably, the proliferative disorder is a cancer or leukaemia. The term proliferative disorder is used herein in a broad sense to include any disorder that requires control of the cell cycle, for example cardiovascular disorders such as restenosis and cardiomyopathy, auto-immune disorders such as glomerulonephritis and rheumatoid arthritis, dermatological disorders such as psoriasis, anti-inflammatory, anti-fungal, antiparasitic disorders such as malaria, emphysema and alopecia. In these disorders, the compounds of the present invention may induce apoptosis or maintain stasis within the desired cells as required.

In a particularly preferred embodiment, the invention relates to the use of one or more compounds of the invention in the treatment of a CDK dependent or sensitive disorder. CDK dependent disorders are associated with an above normal level of activity of one or more CDK enzymes. Such disorders preferably associated with an abnormal level of activity of CDK2 and/or CDK4. A CDK sensitive disorder is a disorder in which an aberration in the CDK level is not the primary cause, but is downstream of the primary metabolic aberration. In such scenarios, CDK2 and/or CDK4 can be said to be part of the sensitive metabolic pathway and CDK inhibitors may therefore be active in treating such disorders. Such disorders are preferably cancer or leukaemic disorders.

A second aspect of the present invention relates to the use of one or more compounds of the invention, and pharmaceutically acceptable salts thereof, in the manufacture of a medicament for use in the treatment of a proliferative disease.

The term "proliferative disorder" has been previously discussed and the same definition applies to the second aspect of the invention.

In a particularly preferred embodiment, the one or more compounds of the invention are administered in combination with one or more other anticancer agents. In such cases, the compounds of the invention may be administered consecutively, simultaneously or sequentially with the one or more other anticancer agents.

As used herein the phrase "manufacture of a medicament" includes the use of a compound of the invention directly as the medicament in addition to its use in a screening programme for further anti-proliferative agents or in any stage of the manufacture of such a medicament.

The compounds of the present invention can be present as salts or esters, in particular pharmaceutically acceptable salts or esters.

Pharmaceutically acceptable salts of the compounds of the invention include suitable acid addition or base salts thereof. A review of suitable pharmaceutical salts may be found in Berge et al, J Pharm Sci, 66, 1-19 (1977). Salts are formed, for example with strong inorganic acids such as mineral acids, e.g. sulphuric acid, phosphoric acid or hydrohalic acids; with strong organic carboxylic acids, such as alkanecarboxylic acids of 1 to 4 carbon atoms which are unsubstituted or substituted (e.g., by halogen), such as acetic acid; with saturated or unsaturated dicarboxylic acids, for example oxalic, malonic, succinic, maleic, fumaric, phthalic or tetraphthalic; with hydroxycarboxylic acids, for example ascorbic, glycolic, lactic, malic, tartaric or citric acid; with aminoacids, for example aspartic or glutamic acid; with benzoic acid; or with organic sulfonic acids, such as ($C_1$-$C_4$)-alkyl- or aryl-sulfonic acids which are unsubstituted or substituted (for example, by a halogen) such as methane- or p-toluene sulfonic acid.

Esters are formed either using organic acids or alcohols/hydroxides, depending on the functional group being esterified. Organic acids include carboxylic acids, such as alkanecarboxylic acids of 1 to 12 carbon atoms which are unsubstituted or substituted (e.g., by halogen), such as acetic acid; with saturated or unsaturated dicarboxylic acid, for example oxalic, malonic, succinic, maleic, fumaric, phthalic or tetraphthalic; with hydroxycarboxylic acids, for example ascorbic, glycolic, lactic, malic, tartaric or citric acid; with aminoacids, for example aspartic or glutamic acid; with benzoic acid; or with organic sulfonic acids, such as ($C_1$-$C_4$)-alkyl- or aryl-sulfonic acids which are unsubstituted or substituted (for example, by a halogen) such as methane- or p-toluene sulfonic acid. Suitable hydroxides include inorganic hydroxides, such as sodium hydroxide, potassium hydroxide, calcium hydroxide, aluminium hydroxide. Alcohols include alkanealcohols of 1-12 carbon atoms which may be unsubstituted or substituted, e.g. by a halogen).

In all aspects of the present invention previously discussed, the invention includes, where appropriate all enantiomers and tautomers of compounds of invention. The man skilled in the art will recognise compounds that possess an optical properties (one or more chiral carbon atoms) or tautomeric characteristics. The corresponding enantiomers and/or tautomers may be isolated/prepared by methods known in the art.

The invention furthermore relates to the compounds of, or of use, in the present invention in their various crystalline forms, polymorphic forms and (an)hydrous forms. It is well established within the pharmaceutical industry that chemical compounds may be isolated in any of such forms by slightly varying the method of purification and or isolation form the solvents used in the synthetic preparation of such compounds.

The invention further includes the compounds of, or of use, in the present invention in prodrug form. Such prodrugs are generally compounds of the invention wherein one or more appropriate groups have been modified such that the modification may be reversed upon administration to a human or mammalian subject. Such reversion is usually performed by an enzyme naturally present in such subject, though it is possible for a second agent to be administered together with such a prodrug in order to perform the reversion in vivo. Examples of such modifications include ester (for example, any of those described above), wherein the reversion may be carried out be an esterase etc. Other such systems will be well known to those skilled in the art.

The present invention also encompasses pharmaceutical compositions comprising the compounds of the invention. In this regard, and in particular for human therapy, even though the compounds of the present invention (including their pharmaceutically acceptable salts, esters and pharmaceutically acceptable solvates) can be administered alone, they will generally be administered in admixture with a pharmaceutical carrier,

excipient or diluent selected with regard to the intended route of administration and standard pharmaceutical practice.

Thus, the present invention also relates to pharmaceutical compositions comprising one or more compounds of the invention or pharmaceutically acceptable salts or esters thereof, together with at least one pharmaceutically acceptable excipient, diluent or carrier.

By way of example, in the pharmaceutical compositions of the present invention, the compounds of the invention may be admixed with any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilising agent(s). Examples of such suitable excipients for the various different forms of pharmaceutical compositions described herein may be found in the "Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller.

The pharmaceutical compositions of the present invention may be adapted for oral, rectal, vaginal, parenteral, intramuscular, intraperitoneal, intraarterial, intrathecal, intrabronchial, subcutaneous, intradermal, intravenous, nasal, buccal or sublingual routes of administration.

For oral administration, particular use is made of compressed tablets, pills, tablets, gellules, drops, and capsules. Preferably, these compositions contain from 1 to 250 mg and more preferably from 10-100 mg, of active ingredient per dose.

Other forms of administration comprise solutions or emulsions which may be injected intravenously, intraarterially, intrathecally, subcutaneously, intradermally, intraperitoneally or intramuscularly, and which are prepared from sterile or sterilisable solutions. The pharmaceutical compositions of the present invention may also be in

form of suppositories, pessaries, suspensions, emulsions, lotions, ointments, creams, gels, sprays, solutions or dusting powders.

An alternative means of transdermal administration is by use of a skin patch. For example, the active ingredient can be incorporated into a cream consisting of an aqueous emulsion of polyethylene glycols or liquid paraffin. The active ingredient can also be incorporated, at a concentration of between 1 and 10% by weight, into an ointment consisting of a white wax or white soft paraffin base together with such stabilisers and preservatives as may be required.

Injectable forms may contain between 10 - 1000 mg, preferably between 10 - 250 mg, of active ingredient per dose.

Compositions may be formulated in unit dosage form, i.e., in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

A person of ordinary skill in the art can easily determine an appropriate dose of one of the instant compositions to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In an exemplary embodiment, one or more doses of 10 to 150 mg/day will be administered to the patient for the treatment of malignancy.

The pharmaceutical compositions of the invention may further comprise one or more additional anticancer agents, for example, existing anticancer drugs available on the market.

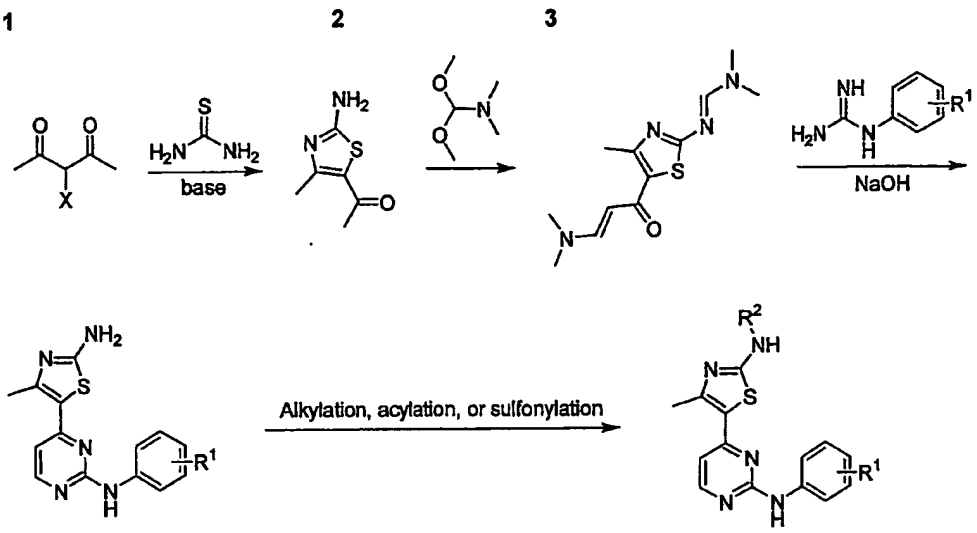
Anticancer drugs in general are more effective when used in combination. In particular, combination therapy is desirable in order to avoid an overlap of major toxicities, mechanism of action and resistance mechanism(s). Furthermore, it is also desirable to administer most drugs at their maximum tolerated doses with minimum time intervals between such doses. The major advantages of combining chemotherapeutic drugs are that it may promote additive or possible synergistic effects through biochemical interactions and also may decrease the emergence of resistance in early tumor cells which would have been otherwise responsive to initial chemotherapy with a single agent. An example of the use of biochemical interactions in selecting drug combinations is demonstrated by the administration of leucovorin to increase the binding of an active intracellular metabolite of 5-fluorouracil to its target, thymidylate synthase, thus increasing its cytotoxic effects.

Numerous combinations are used in current treatments of cancer and leukemia. A more extensive review of medical practices may be found in "Oncologic Therapies" edited by E. E. Vokes and H. M. Golomb, published by Springer.

Beneficial combinations may be suggested by studying the growth inhibitory activity of the test compounds with agents known or suspected of being valuable in the treatment of a particular cancer initially or cell lines derived from that cancer. This procedure can also be used to determine the order of administration of the agents, i.e. before, simultaneously, or after delivery. Such scheduling may be a feature of all the cycle acting agents identified herein.

Suitable anti-proliferative agents that may be used in combination with at least one compound of the present invention include: DNA damaging agents, anti-metabolites, anti-tumour antibiotics, natural products and their analogues, dihydrofolate reductase inhibitors, pyrimidine analogues, purine analogues, cyclin-dependent kinase inhibitors, thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, anthracyclines, vinca drugs, mitomycins, bleomycins, cytotoxic nucleosides, pteridine drugs, diynenes, podophyllotoxins, platinum containing drugs, differentiation inducers, and taxanes. Particularly useful members of those classes include, for example, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, tri-substituted purines such as olomoucine, roscovitine, bohemine and purvalanol, flavopiridol, staurosporin, cytosine arabinoside, melphalan, leurosine, actinomycin, daunorubicin, doxorubicin, mitomycin D, mitomycin A, carninomycin, aminopterin, tallysomycin, podophyllotoxin (and derivatives thereof), etoposide, cisplatin, carboplatinum, vinblastine, vincristine, vindesin, paclitaxel, docetaxel, taxotere retinoic acid, butyric acid, acetyl spermidine, tamoxifen, irinotecan and camptothecin. Most preferably the drug moiety is selected from methotrexate, podophyllotoxin (and derivatives thereof), etoposide, camptothecin, paclitaxel, doxorubicin, roscovitine and bohemine.

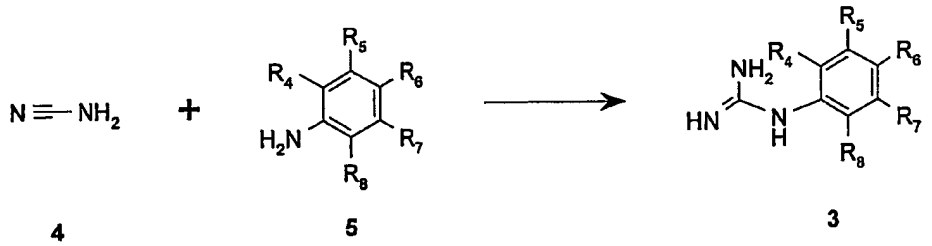
By way of example, the compounds of the invention can be synthesised, by the route shown below in Scheme 1:




*Scheme 1*

As illustrated, acrylate **2** is obtained from heterocyclic methyl ketone **1** by condensation with dimethylformamide dimethylacetal.

Guanidine **3** (*Scheme 2*) can be elaborated by a number of methods known in the art. For the purposes of this invention, the most useful route is amination of cyanamide **4** with anilines **5**.




*Scheme 2*

The present invention is further described by way of example.

## Examples

### Abbreviations

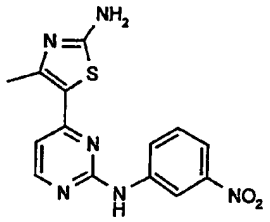
DE MALDI-TOF MS, delayed extraction matrix assisted laser desorption ionisation time-of-flight mass spectrometry; DMF, N,N-dimethylformamide; NMR, nuclear magnetic resonance spectroscopy; RP-HPLC, reversed-phase high performance liquid chromatography; r.t., room temperature; PE, petroleum ether (40-60 °C boiling fraction); DMSO, dimethylsulfoxide.

### General

NMR spectra were recorded using a Varian INOVA 500 MHz instrument. Chemical shifts are reported in ppm (δ) from tetramethylsilane. Silica gel 60 (0.040-0.063 mm) was used for column chromatography.

### Example 1

*[4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine*



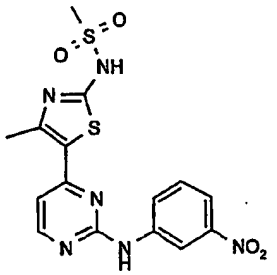

A mixture of thiourea (5.18 g, 0.068 mol) in dry MeOH (20 mL) was stirred and cooled on an ice bath. Pyridine (2 mL) was added, followed by 3-chloro-2,4-pentadione (9.15 g, 0.068 mol) dropwise. After completion of the addition the reaction mixture was allowed to warm to r. t. and stirring was continued for 4 h. The precipitates were filtered and washed with EtOAc to afford white solid 1-(2-amino-4-methyl-thiazol-5-yl)-ethanone.

A solution of this material (3.35 g, 0.021 mol) in *N,N*-dimethylformamide dimethylacetal (10 mL) was refluxed under $N_2$ for 4 – 6 h. The reaction mixture was evaporated to dryness. EtOAc was added to the residue and the precipitates were collected by filtration and were washed with EtOAc/PE (5:1, v/v) to afford *N'*-[5-(3-dimethylamino-acryloyl)-4-methyl-thiazol-2-yl]-*N,N*-dimethyl-formamidine as an orange solid (50 – 79 %). $^1$H-NMR ($CDCl_3$) $\delta$: 2.64 (s, 3H, $CH_3$), 3.08 (s, 6H, $CH_3$), 3.11 (s, 6H, $CH_3$), 5.35 (d, 1H, $J$ = 12.2 Hz, CH), 7.67 (d, 1H, $J$ = 12.2 Hz, CH), 8.23 (s, 1H, N=CH). DE MALDI-TOF MS: $[M+H]^+$ = 267.49 ($C_{12}H_{18}N_6OS$ requires 266.36).

A mixture of this material (2.19 g, 8.2 mmol) and 3-nitrophenyl guanidine nitrate (2.00 g 8.2 mmol) in 2-methoxyethanol (10 mL) was treated with NaOH (0.33 g). After refluxing under $N_2$ for 20 h the reaction mixture was concentrated and purified by silica-gel chromatography using EtOAc/PE (7:1) to elute the title compound as a light-yellow solid (1.95 g, 72 %), which was then recrystallised from EtOAc/MeOH. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.13 (s, 3H, $CH_3$), 7.02 (d, 1H, $J$ = 5.5 Hz, Py-H), 7.59 (m, 4H, Ph-H and $NH_2$), 7.82 (m, 1H, Ph-H), 8.16 (m, 1H, Ph-H), 8.44 (d, 1H, $J$ = 5.5 Hz, Py-H), 8.86 (br. s, 1H, NH).

Example 2

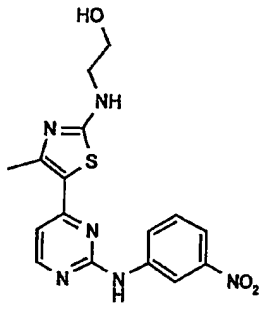
*N-{4-Methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-yl}-methanesulfonamide*



To a mixture of [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine (0.33 g, 1.0 mmol) and methylsulfonyl chloride (0.22 g, 2.0 mmol) in dry DMF (2 mL) was added $Et_3N$ (0.28 mL). The reaction mixture was stirred at r. t. for 20 h. The product was isolated as a yellow solid by preparative RP-HPLC (Vydac 218TP1022, 9 mL/min) using a gradient from 10 – 70 % MeCN in 0.1 % aq $CF_3COOH$ over 40 min. Anal. RP-HPLC: $t_R$ = 17.40 min (Vydac 218TP54, 0 – 60 % MeCN in 0.1 % aq $CF_3COOH$ over 20 min, 1 mL/min, 25 °C, purity > 97 %). $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.10 (s, 3H, $CH_3$), 3.25 (s, 3H, $CH_3$), 7.05 (d, 1H, $J$ = 5.2 Hz, Py-H), 7.42 (m, 1H, Ph-H), 7.63 (m, 1H, Ph-H), 7.98 (m, 1H, Ph-H), 8.21 (d, 1H, $J$ = 5.2 Hz, Py-H), 8.42 (s, 1H, Ph-H), 9.18 (s, 1H, NH).

Example 3

*2-{4-Methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-ylamino}-ethanol*

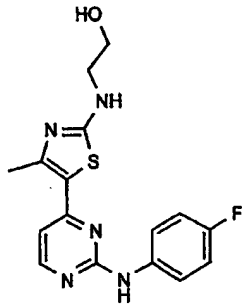



To a mixture of [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine (0.33 g, 1.0 mmol) and iodoethanol (0.44 g, 2.6 mmol) in dry DMF (2 mL) was added *tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3-diazaphosphorine (0.5 mL). The reaction mixture was heated at 124 °C for 20 h. The product was isolated as a brown solid by preparative RP-HPLC (Vydac 218TP1022, 9 mL/min) using a gradient from 10 – 70 % MeCN in 0.1 % aq $CF_3COOH$ over 40 min. Anal. RP-HPLC: $t_R$ = 14.30 min (Vydac 218TP54, 0 – 60 % MeCN in 0.1 % aq $CF_3COOH$ over 20 min, 1 mL/min, 25 °C, purity > 97 %). $^1$H-NMR ($CD_3OD$) $\delta$: 3.30 (s, 3H,

$CH_3$), 3.91 (t, 2H, $J$ = 4.6 Hz, $CH_2$), 4.25 (t, 2H, $J$ = 4.6 Hz, $CH_2$), 7.21(d, 1H, $J$ = 5.2 Hz, Py-H), 7.54 (m, 1H, Ph-H), 7.89 (m, 2H, Ph-H), 8.59 (d, 1H, $J$ = 5.2 Hz, Py-H), 8.90 (s, 1H, Ph-H).

Example 4

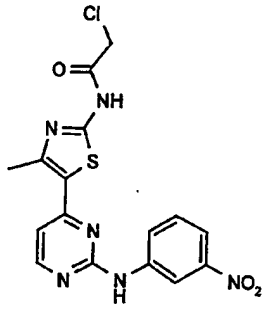
*2-{5-[2-(4-Fluoro-phenylamino)-pyrimidin-4-yl]-4-methyl-thiazol-2-ylamino}-ethanol*




This compound was prepared from [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine in a manner analogous to that described in Example 3. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.44 (s, 3H, $CH_3$), 3.54 (m, 2H, $CH_2$), 4.78 (m, 2H, $CH_2$), 6.87 (d, 1H, $J$ = 5.2 Hz, Py-H), 7.09 (m, 2H, Ph-H), 7.75 (m, 2H, Ph-H), 8.30 (d, 1H, $J$ = 5.2 Hz, Py-H), 8.11 (m, 1H, NH), 9.43 (s, 1H, NH). DE MALDI-TOF MS: $[M+H]^+$ = 345.79 ($C_{16}H_{16}FN_5OS$ requires 345.40).

Example 5

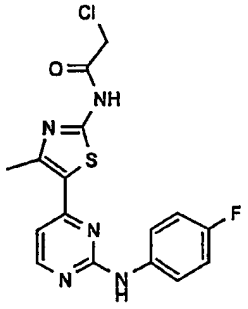
*2-Chloro-N-{4-methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-yl}-acetamide*



A solution of [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine (0.33 g, 1.0 mmol) in dry DMF (3 mL) was cooled on an ice-water bath. Chloroacetyl chloride (0.22 g, 2.0 mmol) and pyridine (80 µL) were added. After stirring at r. t. for 18 h, the product was isolated as a brown solid by preparative RP-HPLC (Vydac 218TP1022, 9 mL/min) using a gradient from 10 – 70 % MeCN in 0.1 % aq $CF_3COOH$ over 40 min. Anal. RP-HPLC: $t_R$ = 20.62 min (Vydac 218TP54, 0 – 60 % MeCN in 0.1 % aq $CF_3COOH$ over 20 min, 1mL/min, 25 °C, purity > 97 %). $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.45 (s, 3H, $CH_3$), 4.12 (s, 2H, $CH_2$), 7.03(d, 1H, $J$ = 5.2 Hz, Py-H), 7.42 (m, 1H, Ph-H), 7.63 (m, 1H, Ph-H), 8.01 (m, 1H, Ph-H), 8.41 (d, 1H, $J$ = 5.2 Hz, Py-H), 8.64 (s, 1H, Ph-H).

Example 6

*2-Chloro-N-{5-[2-(4-fluoro-phenylamino)-pyrimidin-4-yl]-4-methyl-thiazol-2-yl}-acetamide*




This compound was prepared from [4-(2-amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine in a manner analogous to that described in Example 5. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.94 (s, 3H, $CH_3$), 4.75 (s, 2H, $CH_2$), 7.44(m, 3H, Py-H and Ph-H), 8.09 (m, 2H, Ph-H), 8.28 (s, 1H, NH), 8.80 (d, 1H, $J$ = 5.2 Hz, Py-H).

The biological activity of the compounds of the invention was demonstrated by measuring the CDK inhibition by virtue of an assay-based screen, and/or by a cytotoxicity assay using one or more cell lines.

Example 7

Kinase specificity of selected compound

Selected compounds from the above examples were investigated for their kinase selectivity. A panel of protein kinases, including the CDKs relevant to the present invention, as well as a representative number of functionally unrelated kinases, were used.

Assays for CDK4/Cyclin D1, CDK2/Cyclin E, CDIK1/Cyclin B kinase may be carried out by monitoring phosphorylation of GST-Rb in an appropriate system. Thus, GST-Rb phosphorylation, induced by CDK4/Cyclin D1, CDK2/Cyclin E or CDK1/Cyclin

B is determined by incorporation of radio-labeled phosphate in GST-Rb(772-928) using radiolabelled ATP in 96-well format *in vitro* kinase assay. The phosphorylation reaction mixture (total volume 40 µl) consisted of 50 mM HEPES pH 7.4, 20 mM $MgCl_2$, 5 mM EGTA, 2 mM DTT, 20 mM β-glycerophosphate, 2 mM NaF, 1 mM $Na_3VO_4$, Protease Inhibitors Cocktail (Sigma, see above), BSA 0.5mg/ml, 1 µg purified enzyme complex, 10 µl of GST-Rb-Sepharose beads, 100 µM ATP, 0.2µCi $^{32}$P-ATP. The reaction is carried out for 30 min at 30°C at constant shaking. At the end of this period 100 µl of 50 mM HEPES, pH 7.4 and 1 mM ATP is added to each well and the total volume transferred onto GFC filtered plate. The plate is washed 5 times with 200 µl of 50 mM HEPES, pH 7.4 and 1 mM ATP. To each well were added 50 µl scintillant liquid and the radioactivity of the samples is measured on Scintilation counter (Topcount, HP). The IC50 values of different peptides were calculated using GraFit software.

Alternatively, CDK2/cyclin A kinase assays may be performed in 96-well plates using recombinant CDK2/cyclin A. Assay buffer consisted of 25 mM ß-glycerophosphate, 20 mM MOPS, 5 mM EGTA, 1 mM DTT, 1mM $NaVO_3$, pH 7.4, into which is added 2 – 4 µg of CDK2/cyclin A with substrate pRb(773-928). The reaction is initiated by addition of Mg/ATP mix (15mM $MgCl_2$, 100 µM ATP with 30-50 kBq per well of [γ-$^{32}$P]-ATP) and mixtures incubated for 10 – 30 min, as required, at 30 °C. Reactions were stopped on ice, followed by filtration through p81 filterplates (Whatman Polyfiltronics, Kent, UK). After washing 3 times with 75 mM orthophosphoric acid, plates were dried, scintillant added and incorporated radioactivity measured in a scintillation counter (TopCount , Packard Instruments, Pangbourne, Berks, UK).

PKCα kinase activity may be measured by the incorporation of radio-labeled phosphate in Histone 3, as described. The reaction mixture (total volume 65 µl) consist of 50 mM Tris-HCl, 1 mM Calcium acetate, 3 mM DTT, 0.03 mg/ml Phosphatidylserine, 2.4 µg/ml PMA, 0.04% NP40, 12 mM Mg/Cl, purified PKCα -

100 ng, Histone 3, 0.2mg/ml, 100 μM ATP, 0.2 μCi [γ-$^{32}$P]-ATP. The reaction is carried over 15 min at 37°C in microplate shaker and is stopped by adding 10 μl 75 mM orthophosphoric acid and placing the plate on ice. 50 μl of the reaction mixture is transferred onto P81 filterplate and after washing off the free radioactive phosphate (3 times with 200 μl 75 mM orthophosphoric acid per well) 50 μl of scintillation liquid (Microscint 40) were added to each well and the radioactivity is measured on Scintillation counter (Topcount, HP).

For use in said assays CDK2 and/or PKC may be obtained from available sources or produced by recombinant methods as described. His–tagged CDK2/Cyclin E and CDK1/Cyclin B may be co-expressed and PKCα singularly expressed in Sf 9 insect cells infected with the appropriate baculovirus constructs. The cells are harvested two days after infection by low speed centrifugation and the proteins purified from the insect cell pellets by Metal-chelate chromatography. Briefly, the insect cell pellet is lysed in Buffer A (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.02% NP40 and 5 mM β-marcaptoethanol, 1 mM NaF. 1 mM $Na_3VO_4$ and Protease Inhibitors Coctail (Sigma) containing AEBSF, pepstatin A, E 64, bestatin, leupeptin) by sonication. The soluble fraction is cleared by centrifugation and loaded onto Ni-NTA-Agarose (Quiagen). Non bound proteins were washed off with 300 mM NaCl, 5-15 mM Imidazole in Buffer A and the bound proteins eluted with 250 mM Imidazole in Buffer A. The purified proteins are extensively dialyzed against Storage buffer (20 mM HEPES pH 7.4, 50 mM NaCl, 2 mM DTT, 1 mM EDTA, 1 mM EGTA, 0.02% NP40, 10% v/v Glycerol) aliquoted and stored at -70°C. PKC-α - 6 x His may be purified the same way but using different buffers- 50 mM NaH2PO4, pH 8.0 and 0.05% Triton X-100 instead of Tris and NP40 respectively.

The results in the Table 1 below show that the compounds in question exhibit a high degree of selectivity for inhibition of CDKs.

Example 8

Anti-proliferative effect of selected compounds

Selected compounds from the above examples were subjected to a standard cellular proliferation assay using a range of different human tumour cell lines. Standard 72-h MTT (thiazolyl blue; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays were performed (Haselsberger, K.; Peterson, D. C.; Thomas, D. G.; Darling, J. L. Anti Cancer Drugs 1996, 7, 331-8; Loveland, B. E.; Johns, T. G.; Mackay, I. R.; Vaillant, F.; Wang, Z. X.; Hertzog, P. J. Biochemistry International 1992, 27, 501-10). Human tumour cell lines were obtained from the ATCC (American Type Culture Collection, 10801 University Boulevard, Manessas, VA 20110-2209, USA).

The results in Table 1 below illustrate the anti-proliferative effect of compounds described in this application.

**Table 1**

| Compound | Inhibition of kinase activity, $IC_{50}$ (μM) | | *In vitro* anti-proliferative activity, 72-h MTT assay $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|
| | *CDK2 cyclin E* | *CDK4 cyclin D1* | *A549* | HT-29 | *Saos-2* |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine | 0.0002 | 0.41 | 0.22 | 0.34 | 0.42 |
| *N*-{4-Methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-yl}-methanesulfonamide | 0.005 | 1.1 | 0.07 | 0.17 | 0.09 |
| 2-{4-Methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-ylamino}-ethanol | 0.004 | 0.11 | 0.007 | 0.09 | 0.04 |
| 2-{5-[2-(4-Fluoro-phenylamino)-pyrimidin-4-yl]-4-methyl-thiazol-2-ylamino}-ethanol | 0.54 | 15 | 1.3 | 1.4 | 1.8 |
| 2-Chloro-*N*-{4-methyl-5-[2-(3-nitro-phenylamino)-pyrimidin-4-yl]-thiazol-2-yl}-acetamide | 0.24 | 0.44 | 0.14 | 0.14 | 0.17 |
| 2-Chloro-*N*-{5-[2-(4-fluoro-phenylamino)-pyrimidin-4-yl]-4-methyl-thiazol-2-yl}-acetamide | 0.23 | N/d | N/d | N/d | N/d |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-iodo-phenyl)-amine | 0.05 | 0.24 | 0.35 | 0.36 | 0.61 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-bromo-phenyl)-amine | 0.28 | 0.26 | 0.82 | 0.66 | 0.81 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-nitro-phenyl)-amine | 0.18 | 50.00 | 12.31 | 8.61 | 12.92 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine | 0.24 | 0.72 | 1.76 | 1.95 | 2.15 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-iodo-phenyl)-amine | 0.33 | 2.65 | 2.80 | 2.47 | 2.35 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-methoxy-phenyl)-amine | 0.34 | 0.10 | 1.82 | 1.66 | 4.39 |

| Compound | Inhibition of kinase activity, $IC_{50}$ (μM) | | *In vitro* anti-proliferative activity, 72-h MTT assay $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|
| | *CDK2 cyclin E* | *CDK4 cyclin D1* | *A549* | HT-29 | *Saos-2* |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(4-bromo-phenyl)-amine | 1.01 | 1.61 | 2.50 | 2.98 | 1.47 |
| 4-[4-(2,4-Dimethyl-thiazol-5-yl)-pyrimidin-2-ylamino]-benzenesulfonic acid | 0.30 | 5.61 | 100 | 100 | 100 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-chloro-phenyl)-amine | 0.018 | 0.07 | 0.41 | 0.40 | 0.81 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-(3-trifluoromethyl-phenyl)-amine | 0.68 | 5.83 | 1.03 | 1.26 | 0.65 |
| [4-(2-Amino-4-methyl-thiazol-5-yl)-pyrimidin-2-yl]-phenyl-amine | 0.93 | 0.68 | 1.26 | 1.21 | 1.75 |

Various modifications and variations of the described methods of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Modifications of the described modes for carrying out the invention which are obvious to those skilled in the relevant are, or related fields, are thus intended to fall within the scope of the following claims.

CLAIMS

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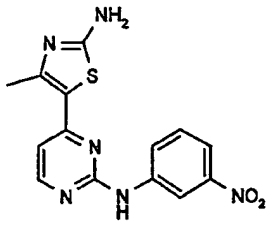

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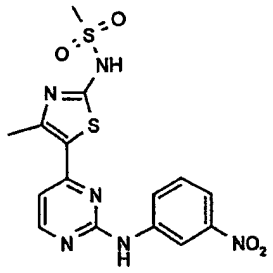

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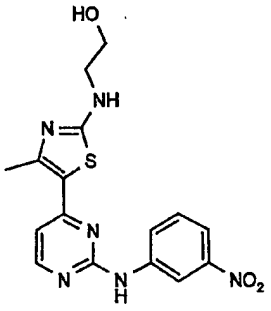

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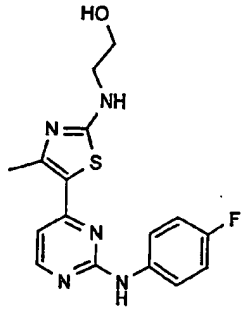

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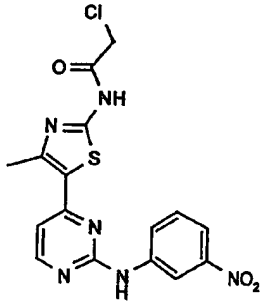

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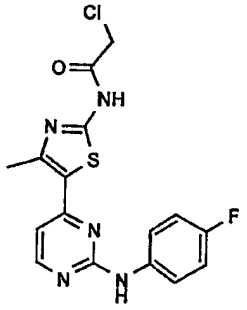

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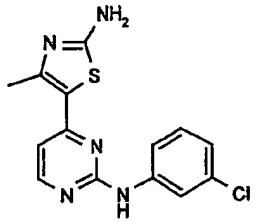

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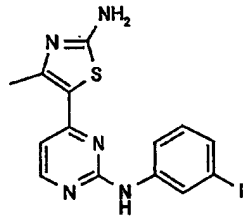

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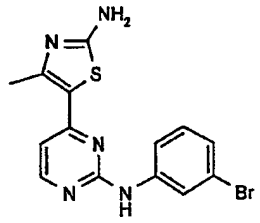

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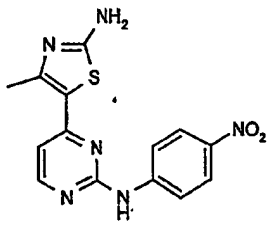

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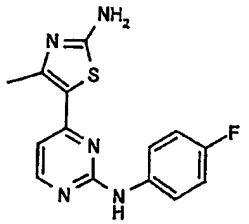

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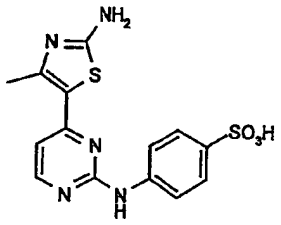

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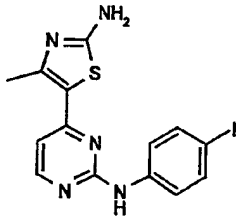

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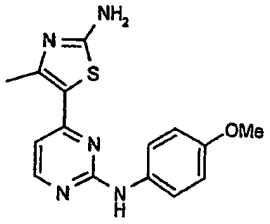
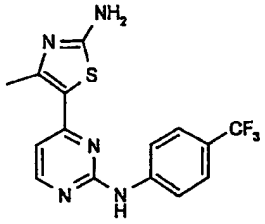
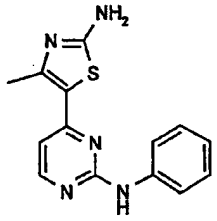


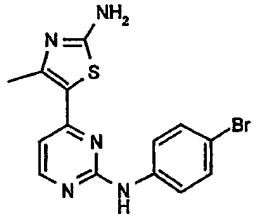
15. A compound having the formula:



16. A compound having the formula:



17. A compound having the formula:



18. A pharmaceutical composition comprising a compound according to any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent, excipient or carrier.

19. A pharmaceutical composition according to claim 18 which further comprises one or more other anticancer agents.

20. Use of one or more compounds as defined in any of claims 1 to 17, or a pharmaceutically acceptable salt thereof, in the treatment of a proliferative disorder.

21. Use according to claim 20 wherein the proliferative disorder is cancer or leukaemia.

22. Use according to claim 20 or claim 21 wherein said one or more compounds are administered in an amount sufficient to inhibit at least one CDK enzyme.

23. Use according to claim 22 wherein the CDK enzyme is CDK2 and/or CDK4.

24. Use of a compound according to any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for use in the treatment of a proliferative disorder.

25. Use according to claim 24 wherein said compound is administered in combination with one or more other anticancer agents.

ABSTRACT

The present invention relates to 2-substituted 4-heteroaryl-pyrimidines, their preparation, pharmaceutical compositions containing them and their use as inhibitors of cyclin-dependent kinases (CDKs) and hence their use in the treatment of proliferative disorders such as cancer, leukaemia, psoriasis and the like.

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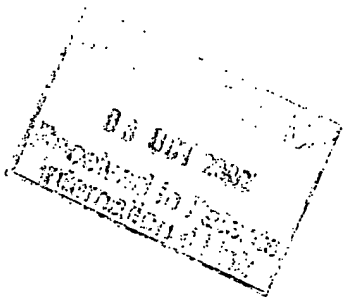

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